

Serial No.: 09/800,700
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REMARKS

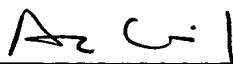
Claims 1-58 are pending in the application. Support for new claims 31-58 is found throughout the specification, for example, see Figures 5A-5R, and pages 5-6 of the specification. Therefore, no new matter has been added.

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

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Dated: 1/29/02



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PENDING CLAIMS

Showing original claims filed with the application ("Original"), claims added by preliminary amendment filed November 26, 2001 ("Added"), and new claims added herein by supplementary preliminary amendment ("New").

1. **(Original)** A fusion polypeptide comprising from the N-terminus:
 - a) a C-terminal intein motif;
 - b) a peptide; and
 - c) an N-terminal intein motif.
2. **(Original)** A fusion polypeptide according to claim 1 wherein said intein has altered cyclization activity as compared to the wild-type intein.
3. **(Original)** A fusion polypeptide according to claim 1 wherein said peptide is a random peptide.
4. **(Original)** A fusion polypeptide according to claim 1 wherein said peptide is derived from a cDNA library.
5. **(Original)** A fusion polypeptide according to claim 1 further comprising a reporter protein.
6. **(Original)** A fusion polypeptide according to claim 5 wherein said reporter protein is fluorescent protein selected from the group consisting of green fluorescent protein, blue fluorescent protein, yellow fluorescent protein, and red fluorescent protein.
7. **(Original)** A fusion polypeptide according to claim 5 wherein said reporter protein is a transcription factor.
8. **(Original)** A fusion polypeptide according to claim 1 further comprising a fusion partner.
9. **(Original)** A library of fusion polypeptides according to claim 1 or 6.
10. **(Original)** A fusion nucleic acid comprising from 5' to 3':
 - a) nucleic acid encoding a C-terminal intein motif;
 - b) nucleic acid encoding a peptide; and
 - c) nucleic acid encoding an N-terminal intein motif.
11. **(Original)** A retroviral vector comprising the fusion nucleic acid of claim 10.

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12. (Original) A method of making a cyclic peptide in vivo comprising providing a cell comprising a fusion nucleic acid comprising from 5' to 3':

- a) nucleic acid encoding a C-terminal intein motif;
- b) nucleic acid encoding a peptide; and
- c) nucleic acid encoding an N-terminal intein motif;

under conditions whereby a cyclic peptide is formed.

13. (Original) A method according to claim 12 further comprising transforming said cell with said fusion nucleic acid.

14. (Original) A method according to claim 12 wherein a library of cells comprising a library of fusion nucleic acids is provided.

15. (Original) A method comprising:

- a) introducing an intein-catalyzed cyclic peptide library into a cell; and
- b) screening for an altered phenotype.

16. (Original) A method for identifying target molecules comprising:

- a) introducing an intein-catalyzed cyclic peptide library into a cell;
- b) screening said cell for an altered phenotype; and
- c) isolating target molecules that bind to the cyclic peptide.

17. (Original) An intein-catalyzed cyclic peptide library comprising:

- a) an intein;
- b) a random peptide of at least 3 amino acids in length; and
- c) a reporter protein.

18. (Original) A library according to claim 17 wherein said intein is a mutant intein with altered cyclization efficiency.

19. (Added) A method of screening for a mutant intein having increased protein cyclization activity as compared to a wild-type intein, the method comprising:

- a) providing a population of fusion polypeptides, wherein each fusion polypeptide comprises, from the N-terminus;
 - 1) a C-terminal intein motif;
 - 2) a protein; and
 - 3) an N-terminal intein motif;

wherein at least one of said C-terminal intein motif and said N-terminal intein motif comprises a mutation as compared to a wild-type intein motif, and

wherein said C-terminal intein motif and said N-terminal motif comprise said mutant intein;

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- b) assaying for increased cyclization activity catalyzed by said mutant intein as compared to said wild-type intein; and
- c) detecting said increased cyclization activity.

20. (Added) The method according to claim 19, wherein said protein comprises a reporter protein.

21. (Added) The method according to claim 20, wherein said reporter protein is Green Fluorescent Protein (GFP).

22. (Added) The method according to claim 21, wherein said cyclization activity activates fluorescence of said Green Fluorescent Protein (GFP), and in the absence of said cyclization activity, said fluorescence is not activated.

23. (Added) The method according to claim 22, wherein said fluorescence is indicative of the amount or efficiency of said cyclization activity.

24. (Added) The method according to claim 23, wherein said fusion polypeptide is expressed in a cell.

25. (Added) The method according to claim 24, wherein said cyclization activity is catalyzed in the cell.

26. (Added) The method according to claim 25, wherein said assaying comprises assaying for an altered cellular phenotype.

27. (Added) The method according to claim 26, wherein said cyclization activity of said mutant intein is 1-10 fold higher than said wild-type intein.

28. (Added) The method according to claim 19, wherein said mutation is generated by random mutagenesis.

29. (Added) A method for making a fusion polypeptide encoding a mutant intein having increased protein cyclization activity as compared to a wild-type intein, the method comprising introducing into a cell a nucleic acid encoding said fusion polypeptide, wherein said fusion polypeptide comprises, from the N-terminus;

- a) a C-terminal intein motif;
- b) a protein; and
- c) an N-terminal intein motif;

wherein at least one of said C-terminal intein motif and said N-terminal intein motif comprises a mutation as compared to said wild-type intein motif, and said cyclization activity results in the cyclization of said protein.

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- 30. (Added)** The method according to claim 29, wherein said protein comprises a reporter protein.
- 31. (New)** The method according to claim 30, wherein the amino acid sequence of said mutant intein comprises SEQ ID NO:1 containing at least one mutation.
- 32. (New)** The method according to claim 31, wherein said mutant intein comprises a mutation at position 389 of said amino acid sequence.
- 33. (New)** The method according to claim 32, wherein said mutation at position 389 comprises an amino acid substitution from R to K.
- 34. (New)** The method according to claim 31, wherein said mutant intein comprises a mutation at position 23 of said amino acid sequence.
- 35. (New)** The method according to claim 34, wherein said mutation at position 23 comprises an amino acid substitution from S to P.
- 36. (New)** The method according to claim 31, wherein said mutant intein comprises a mutation at position 34 of said amino acid sequence.
- 37. (New)** The method according to claim 36, wherein said mutation at position 34 comprises an amino acid substitution from I to V.
- 38. (New)** The method according to claim 31, wherein said mutant intein comprises a mutation at position 320 of said amino acid sequence.
- 39. (New)** The method according to claim 38, wherein said mutation at position 320 comprises an amino acid substitution from D to N.
- 40. (New)** The method according to claim 39, wherein said amino acid sequence comprises a mutation at position 389.
- 41. (New)** The method according to claim 40, wherein said mutation at position 389 comprises an amino acid substitution from R to K.
- 42. (New)** The method according to claim 39, wherein said amino acid sequence comprises a mutation at position 34.
- 43. (New)** The method according to claim 42, wherein said mutation at position 34 comprises an amino acid substitution from I to V.

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- 44. (New)** The method according to claim 39, wherein said amino acid sequence comprises a mutation at position 36.
- 45. (New)** The method according to claim 44, wherein said mutation at position 36 comprises an amino acid substitution from T to A.
- 46. (New)** The method according to claim 39, wherein said amino acid sequence comprises a mutation at position 23.
- 47. (New)** The method according to claim 46, wherein said mutation at position 23 comprises an amino acid substitution from S to P.
- 48. (New)** The method according to claim 39, wherein said amino acid sequence comprises a mutation at position 369.
- 49. (New)** The method according to claim 48, wherein said mutation at position 369 comprises an amino acid substitution from K to R.
- 50. (New)** The method according to claim 30, wherein the amino acid sequence of said mutant intein comprises SEQ ID NO: 32.
- 52. (New)** The method according to claim 30, wherein the amino acid sequence of mutant intein comprises SEQ ID NO: 34.
- 53. (New)** The method according to claim 31, wherein the amino acid sequence of mutant intein comprises SEQ ID NO: 36.
- 54. (New)** The method according to claim 31, wherein the amino acid sequence of mutant intein comprises SEQ ID NO: 38.
- 55. (New)** The method according to claim 31, wherein the amino acid sequence of mutant intein comprises SEQ ID NO: 40.
- 56. (New)** The method according to claim 31, wherein the amino acid sequence of mutant intein comprises SEQ ID NO: 42.
- 57. (New)** The method according to claim 31, wherein the amino acid sequence of mutant intein comprises SEQ ID NO: 44.
- 58. (New)** The method according to claim 31, wherein the amino acid sequence of mutant intein comprises SEQ ID NO: 46.